

Animal Toxicology

by R. Kimbrough,* J. Buckley,† L. Fishbein,‡
G. Flamm,** L. Kasza,** W. Marcus,†† S. Shibko,**
and R. Teske**

Introduction

As a number of reviews on different aspects of the toxicology of the PCBs have been written recently (1-4) no effort has been made in this report to give a complete outline of the animal toxicology.

The material summarized in this report consists predominantly of more recently published information on toxic effects of PCBs in mammals and experimental studies in birds. Since Nelson et al. (3) discussed extensively the toxic effects produced in fish by PCBs, the reader is referred to this and the other reviews cited above for information on toxicity of PCBs in this species.

The emphasis of the present review was placed on chronic toxic effects wherever possible. Unfortunately, very little information was available for halogenated dibenzofurans and the brominated biphenyl mixtures.

Acute Toxicity

Polychlorinated Biphenyls (PCB)

The acute oral LD₅₀ of the PCBs in rats, rabbits and mice ranges from 1 to 10 g/kg body weight (Tables 1 and 2.) There is some indication that young animals may be more sensitive than adults, and

females more sensitive than males (Table 1). According to the classification used by the American Industrial Hygiene Association (12), the acute toxicity of the PCBs can be classed as slightly toxic (0.5-5 g/kg body weight) to practically nontoxic (5-15 g/kg body weight).

The symptoms associated with administration of a toxic dose of PCB (Aroclor 1242) in rats consists of diarrhea, diminished exploratory behavior, decreased response to pain stimuli, chromodacryorrhea, adipisia, oliguria, anorexia, erythema of limbs, followed by ataxia, coma, and death. Death occurred up to 14 days following administration of the toxic dose. At 24 hr after administration of a single toxic dose of Aroclor 1242, gross pathological examination indicated all organs appeared normal except the liver and kidneys. No inflammation was observed in the abdominal and intestinal mucosa. Histopathological findings showed large discrete sudanophilic vacuoles in the hepatocytes. Widely scattered foci of tubular epithelial cells were present in the kidneys (7). However, Kimbrough (1) reported ulceration of the gastric and duodenal mucosa in rats after single oral doses of 3000 mg/kg Aroclor 1254 or 1260.

Only one metabolite of PCB is known to have been tested for the acute LD₅₀. The 5-OH metabolite of 2,4,3',4'-tetrachlorobiphenyl was more toxic than the parent compound (0.43 g/kg body weight, relative to 2.15 g/kg body weight (Table 1). Dermal toxicity of PCB is summarized in Table 3.

Polybrominated Biphenyls (PBBs)

Polybrominated biphenyls show a low order of acute toxicity to the rat (Table 4). In the case of chicks, a 37% mortality was reported when PBB was administered in the diet at a level of 400 ppm for

* Center for Disease Control, Toxicology Branch, Clinical Chemistry Division, Bureau of Laboratories, Department of Health, Education and Welfare, Public Health Service, Atlanta, Ga. 30333.

† Retired; formerly Environmental Protection Agency, 401 M St., S. W., Washington, D. C. 20460.

‡ National Center for Toxicological Research, Jefferson, Ark. 72079.

†† Environmental Protection Agency, 401 M St., S. W., Washington, D. C. 20460.

** Food and Drug Administration, 200 C St., S. W., Washington, D. C. 20204.

Table 1. Acute toxicity.

Compound tested	Species and sex	Route	LD ₅₀ ^a , g/kg body weight	Reference
Aroclor 1254	Rat (adult, Sherman strain)	Oral	4-10	(5)
Aroclor 1260	Rat (adult, Sherman strain)	Oral	4-10	(5)
Aroclor 1254	Rat (weanling, Sherman strain)	Oral	1.295	(5)
Aroclor 1260	Rat (weanling, Sherman strain)	Oral	1.315	(5)
Aroclor 1254	Rat (female, Sherman strain)	Intravenous	0.358	(5)
Aroclor 1221	Rat (female, Sherman strain)	Oral	4.00	(6)
Aroclor 1262	Rat (female, Sherman strain)	Oral	11.3	(6)
Aroclor 1240	Rat	Oral	4.25	(7)
Aroclor 1254	Rat (Wistar, 30-day-old, M-F)	Oral	1.3	(8)
Aroclor 1254	Rat (Wistar, 60-day-old, M-F)	Oral	1.4	(8)
Aroclor 1254	Rat (Wistar, 120-day-old, M)	Oral	2.0	(8)
Aroclor 1254	Rat (Wistar, 120-day-old, F)	Oral	2.5	(8)
Kaneclor-400	Rat (Wistar, M)	Oral	1.30 (ml/kg)	(9)
Kaneclor-400	Rat (Wistar strain, F)	Oral	1.14 (ml/kg)	(9)
Kaneclor-400	Mice (CFI strain, M)	Oral	1.875 (ml/kg)	(9)
Kaneclor-400	Mice (CFI strain, F)	Oral	1.57 (ml/kg)	(9)
Kaneclor-300	Rat (Wistar strain, M)	Oral	1.15	(9)
Kaneclor-300	Rat (Wistar strain, F)	Oral	1.05	(9)
BP-200 biphenyls of dichloride and below	Mice (dd strain, F)	Oral	6.36	(10)
2,4'-Dichlorobiphenyl	Mice (dd strain, F)	Oral	7.86	(10)
Trichlorobiphenyl	Mice (dd strain, F)	Oral	3.06-4.25	(10)
Biphenyl of trichloride and below	Mice (dd strain, F)	Oral	9.27	(10)
2,4,3',4'-Tetrachlorobiphenyl	Mice (DVI strain)	Intraperitoneal	2.15	(11)
5-OH derivative of 2,4,3'4'- tetrachlorobiphenyl	Mice (CFI strain)	Intraperitoneal	0.43	(11)
2,3,4,3',4'-Pentachlorobiphenyl	Mice (CFI strain)	Intraperitoneal	0.65	(11)

Table 2. Oral LD₅₀ (rat).^a

Compound tested	LD ₅₀ , g/kg body weight
Aroclor 1221 (Undiluted)	3.98
Aroclor 1232 (Undiluted)	4.47
Aroclor 1242 (Undiluted)	8.65
Aroclor 1248 (Undiluted)	11.0
Aroclor 1260 (50% soln in corn oil)	10.0
Aroclor 1262 (50% soln in corn oil)	11.3
Aroclor 1268 (50% soln in corn oil)	10.9

^a Data of Panel on Hazardous Substances (6).Table 3. Skin LD₅₀, rabbits.^a

Compound tested	LD ₅₀ , g/kg body weight
Aroclor 1221 (Undiluted)	2.000-3.169
Aroclor 1232 (Undiluted)	1.26 -2.0
Aroclor 1242 (Undiluted)	0.794-1.269
Aroclor 1248 (Undiluted)	0.794-1.269
Aroclor 1260 (50% soln in corn oil)	1.26 -2.0
Aroclor 1262 (50% soln in corn oil)	1.26 -3.16
Aroclor 1268 (33.3% soln in corn oil)	2.5

^a Data of Panel on Hazardous Substances (6).Table 4. LD₅₀ of polybrominated biphenyls.

Compound	Species	Route	LD ₅₀ , g/kg	Reference
FireMaster BP-6	Rat	Oral	21.5	(13)
Octabromobiphenyl	Rat	Oral	>2	(14)
Octabromobiphenyl	Bobwhite quail	Oral	>12.5	(14)
Hexabromobiphenyl	Rabbit	Dermal	>5	(14)
Octabromobiphenyl	Rabbit	Dermal	>10	(14)

15 days. Of eight guinea pigs fed 50 ppm dietary PBB, seven died (15). Effects noted included cortical atrophy of the thymus, adrenal enlargement, marked depletion of the follicles, and periarteriole lymphocyte sheaths of the spleen.

Subacute and Reproductive Effects of PCBs, PBBs, and Chlorinated Dibenzofurans

Mammalian Subacute Effects of PCBs

In contrast to the acute toxicity (oral and dermal) of the PCBs which is of relatively low order when the substances are administered as a single dose, aspects of subacute toxicity of both the PCBs and

individual chlorinated biphenyls appear to be of far greater concern, exhibiting species sensitivity and cumulative toxic effects following continuous exposure at low levels.

When groups of mice were given toxic rice bran oil containing 1600 ppm PCBs or 5000 ppm of PCB mixture with 48% chlorine, the toxicity response was similar in the two groups (16).

In a group of rats fed 2000 ppm of Phenochlor DP-6, deaths occurred between the 12th and 26th days; enlarged livers, atrophy of the spleens, and a progressively induced hepatic porphyria were noted (17). Administration of 1000 ppm of Aroclor 1254 in the diet of rats resulted in deaths between the 28th day and 53rd day (100% mortality) of feeding (18), 50% mortality at the same dosage in an 8-month study was reported by Kimbrough et al. (19).

Two groups of 52 male Sherman strain weanling rats were fed 100 ppm Aroclor 1242 (6.6–3.89 mg Aroclor/kg body weight) or 100 ppm Aroclor 1016 (6.9–3.5 mg Aroclor/kg body weight) for 6 months, and 54 rats were fed ground chow. Groups of four rats were sacrificed at intervals. Microscopic examination of the liver showed evidence of lipid accumulation, enlarged liver cells, inclusions in a number of livers, and hemorrhage and necrosis in one animal per experimental group. No difference in effect between the two compounds was noted (20).

Rats given 100 mg Aroclor 1242/kg body weight by stomach tube every other day for 3 weeks showed histopathological changes only in the liver and kidneys as foci of sudanophilic vacuolation but no overt signs of toxicity (7).

Rats (Sprague-Dawley) given 100 ppm of 2,5,2',5'-tetrachlorobiphenyl in the diet for 3 weeks showed less liver hypertrophy and fewer biochemical changes than rats given a similar dose of Aroclor 1248 (21).

Administration of 100 mg/kg body weight of Aroclors 1242 or 1254 orally to rabbits once a week for 14 weeks resulted in enlarged livers with apparent destruction of the rough endoplasmic reticulum and atrophy of the uteri, while Aroclor 1221 did not cause this effect (22).

The unusual sensitivity of mink to PCBs was reported by Aulerich et al. (23) who noted 100% mortality within 6 months following administration of 30 ppm PCB (10 ppm each of Aroclor 1242, 1248, and 1254) to adult mink. Feeding of Aroclor 1254 at levels of 5 and 10 ppm in the diet for 4 months led to a dose-dependent retardation of weight gain of growing female mink. Platonow and Karstad (24) produced 100% mortality in mink when they fed this species 3.6 ppm Aroclor 1254 in the diet for 105 days.

Rhesus monkeys are also very sensitive to PCBs (25). For example, adult female rhesus monkeys (*Macaca mulatta*) have been fed diets containing 2.5, 5.0, and 25.0 ppm of Aroclor 1248 over periods ranging from 2 months for the higher PCB levels to 1 year for the two lower doses (26, 27). Animals on the 25 ppm dose developed facial edema, alopecia, and acne within 1 month, and one of six animals had expired as a result of PCB intoxication 2 months after removal from the diet. The total intake of PCBs ranged from 250 to 400 mg/animal. As was the case with the higher doses, these animals developed anemia, hypoproteinemia, bone marrow atrophy and severe hypertrophic hyperplastic gastritis. PCB concentrations in subcutaneous adipose tissue averaged 127 ppm at termination of the experimental diet and 34 ppm 8 months later. Surviving animals continued to show clinical signs and lesions of PCB intoxication 2 years following PCB exposure (28).

When adult rhesus monkeys were fed diets containing 2.5 and 5.0 ppm Aroclor 1248 for 1 year, the females of the group developed periorbital edema, alopecia, erythema and acne from lesions that involved the face and neck within 1 to 2 months (26, 28). Although males consumed more PCB, they exhibited only moderate periorbital edema and erythema. An abnormal dysplastic growth pattern of the gastric mucosa was also noted in male rhesus monkeys fed 300 ppm Aroclor 1248 for 3 months (29).

Cyromegalous or squirrel monkeys fed different dietary levels of Kanechlor 400 showed weight loss, palpebral edema, enlargement of the liver and pneumonia. The lowest effective total dose was 300 mg/kg body weight given over 20–32 weeks (16).

A rhesus monkey fed 3 ppm Aroclor 1242 for 8 months died spontaneously and on autopsy was found to possess a gastric lesion manifested by extensive down growth of the epithelium deep into the subserosa and into the muscular wall of the stomach itself (25).

One monkey fed 2,4,4'-trichlorobiphenyl and another fed 2,4,5,4',5'-pentachlorobiphenyl at 10 ppm for 90 days were reported clinically well, and autopsies showed no positive findings (25, 30).

Third-litter sows received feed containing Aroclor 1242 at a concentration of 20 ppm throughout gestation and nursing (31). Treated sows differed significantly from control sows in the number of live pigs farrowed. Treated sows also had more mummified fetuses. Performance of live-born pigs was not affected by feeding PCB to the sows during nursing. Pathologic changes in the sows included hypertrophy of the liver and erosion of the stomach. Slight atrophy of the spleen and thyroid gland were observed in pigs from treated sows (31).

Dermal toxicity studies in rabbits of technical PCB samples that contained an average of 60% chlorine (Phenoclor DP6, Clophen A60, and Aroclor 1260) as well as fractions containing tetra and pentachlorodibenzofuran have been recently described by Vos and Beems (32). PCB-induced skin lesions were hyperplasia and hyperkeratosis of the epidermal and follicular epithelium following application of 118 mg of the three PCBs (5 times per week for 38 days) on the back skin of adult female New Zealand rabbits. Histopathology of the liver included centrilobular degeneration, centrilobular liver cell atrophy, focal necrosis, and cytoplasmic hyalin degeneration. PCB-induced kidney lesions were hydropic degeneration of the convoluted tubules and tubular dilation with the presence of casts. Definitive hyperplasia and hyperkeratosis of the follicular epithelium of the ear skin were seen after the topical application of fractions of Phenoclor and Clophen, while the fraction from Aroclor caused a minimal hyperplasia and hyperkeratosis of the follicular epithelium. Other effects elicited by the dermal application of the PCBs included thymus atrophy and lymphopenia as well as elevated excretion of fecal coproporphyrin and protoporphyrin. From the response of the back skin and the liver of the rabbit to the three PCB mixtures, and from the response of the ear to the 25% diethyl ether-hexane fractions it was concluded that there were definite quantitative differences in toxicity, at least between the samples used in the above study and prior studies. The extent to which these samples are representative of the normal commercial output has not been established; this situation emphasizes the difficulty in the evaluation of toxicity data of PCBs in which the samples may differ in the amount and nature of toxic impurities.

Vos and Notenboom-Ram (33) compared the toxicity of Aroclor 1260 with a single isomer 2,4,5,2',4',5'-hexachlorobiphenyl in New Zealand rabbits. Dermal applications of a total 120 mg Aroclor 1260 (5 times/week for 28 days) resulted in early macroscopic skin lesions. The lesions in a of rabbits treated similarly with 2,4,5,2',4',5'-hexachlorobiphenyl appeared later and were less severe.

When groups of five male mice were fed dietary levels of 10, 30, 100, and 300 ppm of the PCB isomers 3,4,5,3',4',5'-hexachlorobiphenyl, 2,4,5,2',4',5'-hexachlorobiphenyl, and 2,4,6,2',4',6'-hexachlorobiphenyl for 4 weeks it was noted that the 3,4,5,3',4',5', isomer of hexachlorobiphenyl caused death in mice at a dietary intake of 10 ppm (2.1 mg/kg body weight) within 36–47 days of exposure, while consumption of the other two isomers only resulted in death at a dietary level of 300 ppm (64.3 mg/kg body weight). Body weight was reduced

at the lowest dose for the 3,4,5,3',4',5', isomer and only at the highest dose for the other two isomers. Atrophy of the thymus and hepatomegaly was noted at the lowest dose in the mice receiving the 3,4,5,3',4',5'-isomer, and only this isomer produced experimental porphyria.

Avian Subacute Toxic Effect of PCBs

Chickens fed 10 ppm Aroclor 1242, or 100 ppm Aroclor 1254 showed diminished growth but a dietary level of 100 ppm Aroclor 1260 did not produce this effect (34).

Symptoms of PCB poisoning in birds consist of tremor, ataxia, ruffling, loss of feathers. Edema of the subcutaneous tissues and fluid accumulation of the abdominal and thoracic cavities are characteristic signs at autopsy. These findings are responsible for this disease being designated chick edema disease. Several outbreaks of chick edema disease caused by PCBs have been reported (35–37).

Chicks (one-day-old White Leghorn cockerels) were fed 2,3,6,2',3',6'-hexachlorobiphenyl, 2,4,6,2',4',6'-hexachlorobiphenyl, 2,3,,2',3',4'-hexachlorobiphenyl and 2,4,5,2',4',5'-hexachlorobiphenyl for 21 days at a level of 400 ppm (38). The isomer 2,4,5,2',4',5'-hexachlorobiphenyl was also fed at the dietary level of 100 ppm. The 3,4,5,3',4',5' isomer was the most toxic, causing death of all chicks within 11 days after onset of exposure. These chicks had chick edema disease, atrophy of the thymus and loss of subcutaneous and visceral adipose tissue. The other isomers also caused weight loss, the 2,4,6,2',4',6' isomer being the least effective. Liver enlargement was noted for all groups the 2,3,6,2',3',6' being the least effective and the 2,4,6,2',4',6' the most effective. The microscopic findings made in the liver were most pronounced in the group fed the 2,4,6,2',4',6' isomer. (Since the chicks fed the 3,4,5,3',4',5' isomer died relatively early in the experiment pathological findings in the livers of these chicks are not comparable to those made for the other isomers.)

When chicks were fed 5% rice oil which had produced Yusho they developed chick edema in 17 days. Similar symptoms were produced when chicks were fed 400 ppm Kaneclor 400 (39).

Mammalian Reproductive Effects of PCBs

Oral administration of 0.025 mg/day of Clophen A60 in peanut oil to NMRI-mice for 10 weeks lengthened the estrus cycle from 6.6 ± 2.5 days to 8.7 ± 4.3 days (40, 41). Females treated analogously for 62 days and mated with untreated males exhibited a significant decrease in the implantation rate

from 87.0% in the controls to 79.5%. Male castrated mice were given 0.25 g PCB (Clophen A60) in peanut oil/day by stomach tube for 28 days and 70 μ g testosterone propionate daily on days 19–28. The dry weights of the seminal vesicles after this treatment period was compared to those of vesicles from castrated male mice given testosterone but not PCB and were found to be significantly reduced.

Groups of 10 NMR I female mice were injected subcutaneously with 50 mg/kg body weight of Clophen A60 in peanut oil or the vehicle alone on the day of parturition and thereafter once weekly for 3 weeks. The 209 offspring were bred when reaching sexual maturity by mating them in pairs as follows: 17 pairs of controls, 19 pairs in which the males had received Clophen A60 as a suckling, 23 pairs in which the female had received Clophen A60 as sucklings and 23 pairs in which both sexes had received Clophen A60 as sucklings. The controls had an average of 11.5 offspring per litter while the parents where both mates received PCBs as sucklings had 8.8 offspring per litter. The resorption rate for both groups was the same but the number of implantations was reduced. Reproduction was not affected in those cases in which only one mate received the PCB (42).

Keplinger et al. (43) reported low mating indices and decreased survival of pups for rats receiving Aroclor 1242 at 100 ppm, and decreased survival of pups receiving Aroclor 1254 at 100 ppm. No reproductive effects were found with Aroclor 1260 at 1, 10, or 100 ppm, or with Aroclor 1242 or 1254 at 1 or 10 ppm. These studies suggest that in mammals reproductive effects decrease with increasing chlorination.

Sherman rats were exposed to polychlorinated biphenyls, Aroclor 1254 and Aroclor 1260. Rats exposed to Aroclor 1254 at dietary levels of 20 ppm or more had fewer pups per litter than the controls in the F_{1b} and F_2 generations (5). The 100 ppm exposure level of Aroclor 1254 increased mortality in the F_{1b} offspring and markedly decreased mating performance of the F_{1b} adults. The 500 ppm dietary level of Aroclor 1260 reduced litter size and decreased survival in the F_1 litters. Dietary levels of 5 ppm Aroclor 1254 and 100 ppm Aroclor 1260 had no effect on reproduction in rats exposed through two generations. Liver weights were increased in 12-day old F_1 male weanlings at the 1 ppm level of Aroclor 1254 and in either sex of F_1 and F_2 weanlings at 5 ppm or higher levels of both Aroclor 1254 and 1260. Histological changes in the liver and increased liver weights were observed in adult rats exposed to the higher levels. Pregnant rats given Aroclor 1254 at the rate of 100 mg/kg-day on days 7–15 of gestation produced grossly normal litters, but only 30.1% of

the pups survived to weaning. Reproduction and pup survival were not affected at dosage rates of 50 mg Aroclor 1254/kg body weight per day or 100 mg Aroclor 1260/kg-day.

Rabbits have been found to be more sensitive to PCBs than rats in regard to fetotoxic and reproductive effects (44). In rabbits, no effects were reported at dosages of 6.25 or 10 mg Aroclor 1254/kg-day, but at dosages of 12.5 to 50 mg/kg-day, fetotoxic effects were noted. Abortions, maternal death and stillborns occurred, but no consistent skeletal abnormalities were found.

Ringer et al. (45) found that 1 ppm of Aroclor 1254 caused a slight reduction in reproductive success in mink, but at 5 ppm Aroclor 1254 complete reproductive failure was noted. In the same study, 12 mink were fed a diet containing 30% Coho Salmon from Lake Michigan. None of the mink whelped, while 11 of 12 controls whelped.

Female adult rhesus monkeys fed Aroclor 1248 at a level of 25 ppm for 2 months experienced either fetal resorption or abortion during their second month of pregnancy (evidenced by regression in uterine size and reestablishment of menstruation (26). A third animal carried her fetus to term. This infant's weight was considerably lower than the average rhesus infant (375 g vs. 544 ± 101 g) and had 25 μ g PCB/g of adrenal and adipose tissue. Adult female rhesus monkeys fed 2.5 and 5.0 ppm Aroclor 1248 were bred to control males following the establishment of a relatively steady tissue level of PCB at 6 months. Following 3 matings, 12.5% of the 5 ppm group and 37.5% of the 2.5 ppm group was pregnant compared to 90% in the control group (28). The conception rate of females bred to PCB-fed males was equally as great as that in females bred to control males.

Mammalian Subacute and Reproductive Effects of PBBs

Male Sprague-Dawley rats maintained on diets containing 0, 0.1, 0.01, or 1% octobromobiphenyl for 30 days did not exhibit overt toxicity during this period (14). Gross pathological studies revealed enlarged livers and some kidney changes (petechial hemorrhage, enlargement), while histopathological changes in the 1% group included liver lesions consisting of centrilobular cytoplasmic enlargement and vacuolation and kidney lesions consisting of hyaline degenerative cytoplasmic changes (14). Thyroid hyperplasia was observed in all rats in all test groups.

Rats were fed 100 and 1000 ppm octabromobiphenyl in their diets and then a number were sac-

rificed after 2 and 4 weeks of feeding and after 2, 6, and 18 weeks of recovery on an octabromobiphenyl-free diet (46). The hepatocytes were markedly enlarged in rats fed 100 ppm and 1000 ppm of the PBB after 2 weeks of treatment. Histopathologic changes were characterized by hepatocellular hypertrophy with the pathologic changes localized mostly in the centralobular zone. The cytoplasmic inclusions were more pronounced in the livers of rats fed 1000 ppm than 100 ppm of the PBB. There was some evidence of recovery, since the livers of the 100 ppm level animals returned almost to normal 2 weeks after withdrawal of the compound.

Preliminary 28-day feeding studies in rats with octabromobiphenyl at 100 and 1000 ppm demonstrated liver enlargement, hepatocellular alterations with cytoplasmic inclusions, and accumulative effects of bromine in the fat, liver and muscle (14).

Norris et al. (47) demonstrated hematological changes, liver enlargement, and liver and kidney lesions at all levels of octabromobiphenyl (10, 100, 1000 and 100 ppm) in the diet of rats. When three groups of primagravid rats were fed diets containing 100, 1000, or 10,000 ppm of octabromobiphenyl (BB-8) from day 6 through 15 of pregnancy and the pups delivered on day 20, anasarca was observed in one fetus at each of the two highest levels and gastroschisis was observed in another fetus at each of these levels (48). No other gross effects were observed in either the mothers or the pups and dose-related levels of bromine were found in the fetuses.

When 1 g/kg of octabromobiphenyl was applied in corn oil under occluded conditions to rabbits for 5 hr/day for 10 days over a 2-week period, liver enlargement was found, while at 0.1 g/kg enlargement was absent (46).

Hexabromobiphenyl (FireMaster BP-6) fed to pregnant Sprague-Dawley rats and Swiss/ICR mice (100 and 1000 ppm) resulted in a dose-dependent decrease in mean fetal weight (49). Of 37 pregnant mice 16 were controls, 9 mice received 100 ppm, and 12 received 1000 ppm; of 25 pregnant rats, 12 were controls, 7 rats received 100 ppm, and 6 received 1000 ppm. Exencephaly was produced in the offspring of the mice that received both 100 (3/121) and 1000 ppm (2/174), while cleft palate (4/87) and defective kidneys (2/87) were noted in offspring at the 1000 ppm level. Nonpregnant mice fed 1000 ppm PBBs for 11 days showed marked increase in liver size and weight (49).

Cows from the Halbert herd received feed containing PBB at a level of 2914 ppm at a consumption rate of approximately 15 lb/head/day (200 cows) or 8 lb/head/day (200 cows) for a period of approximately 16 days (50). Early signs of toxicity included anorexia, decreased milk production, increased fre-

quency of urination, lacrimation, some lameness, and shrinking of the udder of recently freshened cows. Upon removal of the contaminated feed appetite improved. Cows bred 4-6 weeks prior to onset came back in heat suggesting early resorptions. Later signs exhibited by the cows included hematomas, abscesses, weight loss (particularly in high producing cows), abnormal hoof growth, rough hair coat, alopecia, thickening of skin, dystocia, lack of udder development at freshening, metritis, and negligible milk production in cows freshening 3-4 months after exposure. Cows also exhibited a lack of wound healing, general weakness, and were highly susceptible to stress. Six months after exposure, nonlactating cows showed depression of appetite, prepartum weakness, failed to develop signs of labor and died without calving. Gross pathology included liver and renal degeneration, and hematomas and abscesses in the peritoneal and thoracic cavities.

Twelve 6 to 18 month-old heifers and bulls were offered the contaminated feed *ad libitum*. Five of the animals were dead within 6 weeks. Younger animals became prostrate, went through a short period of coma and died. Signs and lesions included testicular atrophy, abdominal adhesions, and massive liver abscesses. At 5 months only 2 animals remained alive. These became anorexic, would consume only milk and developed signs of hyperkeratosis over the entire body (50, 51).

The health status of 16 herds of dairy cattle exposed to low levels of polybrominated biphenyl (PBB) was compared with that of 15 control herds. Milk production of the contaminated herd was not significantly changed in 1972, 1973, and 1974, and was not significantly different from that of control herds in the same years. Mortality of adult cows and calves, the percentages of cows culled from the herds because of old age and low production, disease, or sterility, and the general health conditions were similar in the two groups. Serum concentrations of calcium, glucose, and cholesterol in contaminated herds were significantly different from those of the control herds, but the relationship to PBB exposure needs further investigation (52).

A study consisting of an investigation of records and herd history of herds accidentally exposed to PBBs was also done (53). Significant findings included a decrease in milk production of 20 to 50%; severe weight loss at calving time; sterility; decreased growth rate in young animals; evidence of soreness and stiffness; poor response to therapy of common diseases; prolonged wound healing; abnormal hoof growth; calf losses; and malformed calves.

Polybrominated biphenyl was administered to four cows at a dose rate of 10 mg/head/day for 60

days. Although the study was not designed as a toxicology study, retrospective evaluation of the data provided no evidence of clinical problems among test animals during the period of exposure to PBB nor for a year thereafter (54).

Mammalian Toxicity of Chlorinated Dibenzofurans

It is presently assumed because of our experience with the chlorinated dibenzodioxins, that 2,3,7,8-tetrachlorodibenzofuran is the most toxic compound of this group of chloro chemicals, while the dibenzofuran itself or the octadibenzofuran have little inherent toxicity on an acute basis (1).

Bauer et al. (55) in 1961 demonstrated the toxicity of mixtures of tri- and tetrachlorodibenzofurans. A single dose of 1 mg or 0.5 mg/kg body weight given orally to rabbits caused severe and often fatal liver necrosis. Application of this material to the rabbit ear caused severe hyperkeratosis at the application site.

The single oral LD₅₀ for tetrachlorodibenzofuran for guinea pigs is between 5 and 10 µg/kg. Symptoms of toxicity were severe weight loss, atrophy of the thymus and spleen. Hemorrhages were observed in the adrenals, urinary bladder and single cell necrosis was noted in the liver. CD strain rats given as much as 1000 µg/kg body weight of the TCDF failed to show any symptoms of toxicity and microscopic examination of their tissues did not reveal any abnormalities. Similarly, oral single doses of up to 6000 µg/kg body weight of TCDF failed to elicit any toxic effect in C57B1 mice. A subcutaneous dose of 6000 µg/kg given to these mice produced weight loss, hepatomegaly and atrophy of the thymus but no fatalities (56).

While these findings indicate that in mice TCDF is 30-fold less toxic than TCDD, the overall estimated difference in toxicity between TCDD and TCDF is about tenfold (1, 55, 57).

Avian Toxicity of Chlorinated Dibenzofurans

When 1-day-old chicks were started on daily oral doses of 5 µg/kg TCDF daily, most of them died within 11.5 days (56). Of six chicks given daily oral doses of 1 µg/kg, one died. Symptoms of toxicity at the low as well as the high doses consisted primarily of weight loss, decreased food consumption and general failure to thrive. At autopsy the most striking findings were subcutaneous edema, ascites hydropericardium, and atrophy of the thymus. An inflammatory exudate was noticed on microscopic

examination of the lung and the pericardial surface of the heart showed inflammatory changes. Congestion and hemorrhage into the gastrointestinal tract was also observed (56).

Mammalian Toxicity of Brominated Dibenzofurans

When daily doses of 4 µg/rabbit of 2,3,7,8-tetrabromodibenzofurans were applied to the rabbit ear for 5 days, giving a total dose of 20 µg/rabbit or about 5-6 µg/kg body weight, the rabbits developed hyperkeratosis of the treated ear and areas of liver cell necrosis (58). No other information is presently available on the toxicity of brominated dibenzofurans.

Long-Term Toxicity Including Tumorigenesis

Chlorinated Biphenyls: Mice. When male BALB c/J mice were fed Aroclor 1254 for 11 months at a dietary level of 0 or 300 ppm (49.8 mg/kg body weight) 22 of 50 mice in the experimental group and 24 of 50 in the control group survived (59). The livers of the test animals were markedly increased in size, and showed a qualitative increase in porphyrin. A total of 9 experimental mice showed 10 neoplastic nodules (hematomas, hyperplastic nodules) in their liver ranging in size from 0.1 to 0.5 cm in diameter. Other morphological changes observed in the livers of the experimental group including pleomorphism, areas of necrosis, and foci of adenofibrosis. Neither tumors nor the other morphological changes were noted in the controls. Only one of 24 surviving male BALB c/J mice fed Aroclor 1254 for 6 months followed by a control diet for 5 months had a hepatoma (neoplastic nodule) measuring 0.3 cm in diameter.

Ito et al. (60) fed male dd mice 500, 250, 100, and 0 ppm Kanechlor 500, 400, and 300 in the diet. After one year, hepatocellular carcinomas were observed in 5/12 mice fed Kanechlor 500 at a dietary level of 500 ppm; the other seven mice in this group had nodular hyperplasia (neoplastic nodules). No metastases were observed. Feeding α, β, or γ hexachlorocyclohexane (BHC) isomers with or without Kanechlor 500 at a dietary level of 250 ppm to mice for 24 weeks did not produce any tumors in the liver of animals receiving only the PCB (59). Liver tumors were observed in mice receiving 250 ppm α BHC alone or 250 ppm PCB and either 100 or 50 ppm α BHC or 250 or 100 ppm β BHC. Thus PCBs enhanced the tumor development of liver tumors of α or β isomers.

Chlorinated Biphenyls: Rats. Dietary exposure of male Sherman strain rats to levels of 500 ppm Aroclor 1254 (36.4 mg/kg body weight) for 6 months resulted in pronounced lipid accumulation in the liver which persisted for a 10-month observation period following the discontinuation of the dietary exposure to PCBs. Adenofibrosis (cholangiofibrosis), which was observed in the livers of rats ingesting the PCB for 6 months, was still present 10 months later. Whether this lesion is persistent could not definitely be determined since high levels of the higher chlorinated biphenyl isomers were still present in adipose tissue and liver (61). Adenofibrosis is a focal proliferation of glandular epithelium forming ducts often surrounded by extensive fibrosis. This lesion usually occurs concomitantly in rat livers with hepatocellular carcinomas and has been produced by many known hepatocarcinogens. It was first described by Edwards and White (62). The extremely long retention of some PCB isomers in rats was again noted when male Sherman strain rats were fed 100 ppm Aroclor 1254 for 6 months and then allowed to recover for 16 months. A concentration of 4.4 ppm of PCB was present in the liver and 152 ppm in adipose tissue on a wet weight basis (63).

Continued dietary exposure to commercial PCB mixtures in mammals results in their storage in adipose tissue and over an extended period high levels may be attained (20, 64). Blood levels are more a function of the dose, the rate of metabolism, availability of binding sites in blood, chemical characteristics of the compound given, rather than the amount stored in adipose tissue (65).

In another study (63), 200 experimental female Sherman strain rats were fed 100 mg/kg Aroclor 1260 in the diet (11.6–4.3 mg/kg body weight) for approximately 21 months, and 200 female rats were kept as controls. A total of 184 experimental rats and 173 controls survived to sacrifice at 23 months. Hepatocellular carcinomas were observed in 26/184 experimental rats and in 1/173 controls. None of the controls but 146/184 experimental rats had neoplastic nodules within the liver (hyperplastic nodules). Areas of hepatocellular alteration were noted in 28/173 controls and 182/184 experimental rats. The incidence of tumors in other organs was not altered by PCB ingestion and metastases from the liver tumors were not observed (63). Adenofibrosis of the liver was also noted in this study. In reporting these liver tumors the classification developed in a rat liver tumor workshop was followed (66).

Liver tumors (multiple adenomatous nodules) were also induced in 6/10 female Donryu rats with Kanechlor 400 but not in male rats. The dietary exposure varied throughout the study from 38 to 616

ppm (67). The induction of liver tumors by known carcinogens in Sprague-Dawley rats such as 3'-methyl-4-dimethylaminoazobenzene, *N*-2-fluorenylacetamide, and diethylnitrosamine was inhibited by Kanechlor 500 (68).

Groups of 50 male and female Charles River rats were fed dietary levels of 0, 1, 10, and 100 ppm of Aroclor 1242, 1254, and 1260 respectively (69, 70). At 3, 6, and 12 months after onset of the experiment, 5 rats per each group and sex were sacrificed. The surviving rats were sacrificed 24 months after onset of the experiment. The only body weight depression noted was observed in the female rats fed Aroclor 1254. The author reported that important histopathologic changes were only noted in the rats fed the different Aroclors for 24 months.

The liver weights were increased in the male and female rats fed 100 ppm of Aroclor 1260 and 1254 and only in females fed Aroclor 1242 at a dietary level of 100 ppm. It was noted that of a total of 20 surviving male and female rats (10 male and 10 female rats) fed 100 ppm Aroclor 1242 in the diet for 24 months 8/20 rats showed liver lesions called nodular hyperplasia, 2/20 had hepatomas and 1/20 had a cholangiohepatoma.

A total of 27 male and female rats (13 male and 14 female rats) survived to 24 months in the groups of rats fed 100 ppm Aroclor 1254 of these 13/27 had nodular hyperplasia of the liver 4/27 showed hepatomas and 2/27 showed cholangiohepatomas.

Similar findings were made in the total 27 surviving male and female rats fed 100 ppm Aroclor 1260 for 24 months. In these groups 7/27 male and female rats had nodular hyperplasia of the liver. 5/27 had hepatomas, and 2/27 had cholangiohepatomas (70).

Groups of 4 male and 4 female dogs were fed dietary levels of 0, 1, 10, and 100 ppm Aroclor 1242, 1254, and 1260, respectively, for 2 years; 1 female fed 100 ppm Aroclor 1242, 1 male fed 10 ppm Aroclor 1242, 1 female dog fed 100 ppm Aroclor 1254, 1 female fed 10 ppm and 1 male and 1 female fed 100 ppm Aroclor 1260 died. At the dietary level of 100 ppm of either Aroclor 1254 or Aroclor 1260 the body weight gain was slightly decreased for both sexes. Aroclor 1260 also reduced the body weight gain in the females at the dietary level of 10 ppm. In the beagles fed Aroclor 1260 at a dietary level of 100 ppm, the liver weight was increased and the serum alkaline phosphatase was elevated. All but 1 dog that died had either chronic peritonitis or chronic pneumonitis. Many of the dogs fed 10 or 100 ppm of different PCBs showed chronic inflammation of a variety of organs. Male dogs fed Aroclor 1254 showed diffuse hyperplasia of the interstitial cells of the testes and aspermatogenesis (70).

Other Compounds. No long-term studies on decachlorobiphenyl, polybrominated biphenyls, or chlorinated dibenzofurans and chlorinated naphthalenes have been reported.

Immunosuppressive Effects of PCB and PBB

Polychlorinated biphenyls (PCB) have been implicated as immunosuppressants. Friend and Trainer (71) found a decreased resistance to duck hepatitis virus in PCB exposed ducklings. PCB has been shown to produce lymphoid atrophy in rabbits (32, 72), chickens (17, 73), and guinea pigs (15, 74). In addition, PCBs were found to suppress humoral immune responses to several antigens in rabbits (72, 75) and guinea pigs (15, 74) and to suppress cell-mediated immune reactions in guinea pigs (15). In all of these studies the PCBs studied have been commercial mixtures; the role of contaminants such as chlorinated dibenzofurans needs to be considered.

The effect of HBB on the immune system was studied in chickens and guinea pigs (15). In chickens the atrophy of the lymphoid tissue was observed in bursal follicles and in the spleen. It suppressed the humeral immune response in guinea pigs.

Mutagenicity and Teratology

Polychlorinated Biphenyls

The mutagenicity and cytotoxicity of PCBs Aroclor 1242 and 1254 were studied by Green et al. (76, 77). The lack of mutagenic activity was measured by the dominant lethal test was reported for male Osborne-Mendel rats subjected to four different dosage regimens: (1) single oral intubations of 624, 1250 or 2500 mg/kg (for Aroclor 1242); (2) five daily doses of 125 or 250 mg/kg (for Aroclor 1242); 75, 150, or 300 mg/kg (for Aroclor 1254); (3) five daily doses of Aroclor 1254 at 150 mg/kg, then starved overnight prior to mating; (4) chronic feeding for 70 days at a dietary level of 25 or 100 ppm prior to mating (76). In a second study, bone marrow and spermatogonial cells were cytogenetically investigated from Aroclor-treated Osborne-Mendel rats. Aroclor 1242 was given orally at single doses of 1250, 2500, or 5000 mg/kg or as four daily doses of 500 mg/kg-day; Aroclor 1254 was administered orally as five daily doses of 75, 150, or 300 mg/kg-day. No statistically significant increases in chromosomal aberrations were found for the Aroclor-treated rats. As indicated by Green et al. (77), while these studies were negative in regard to chromosomal mutations, they do not entirely rule out the possibility that PCBs may induce point mutations.

To date, there are no known reports in the literature concerning the induction of point mutations by PCBs in laboratory model systems. The observations of Nilson and Ramel (78) in *Drosophila* where no chromosomal breakage was found among Clophen A30- or A50-treated flies, are fully consistent with those of Green et al. (76, 77).

Female NMRI mice, given 0.025 mg/day of Clophen 60 orally, showed evidence of an increased estrus cycle from 6.6 ± 2.5 days to 8.7 ± 4.3 days and a decreased ova implantation rate from 87.0 to 79.5% (39, 40), also demonstrated a decrease in the frequency of implanted ova among young animals nursed on milk containing PCB (Clophen 60). Both the male and the female contributed to the magnitude of the reduction of implantations. The mechanism by which exposure of the developing male to milk containing PCB leads to a reduction in the percent of ova implanted is unknown but need not necessarily involve dominant lethal events.

No reports of terata were found, but a number of studies indicated transplacental passage of PCBs. Grant et al. (79) showed that the concentration of PCB in fetal tissue from oral administration of Aroclor 1254 was dose-dependent and that accumulation occurred in fetal liver where higher concentrations were found than in maternal liver. Placental transport of PCB has been reported for the rabbit, rat, mouse, and cow (79-83). Evidence of placental transfer was also observed among Yusho patients. While PCB has no known or clearly defined teratogenic effect in mammals, their easy passage across the placental suggests the possibility of potential for some form of fetal toxicity.

Brominated Biphenyls

Pregnant rats (15) were force-fed 100 mg hexabromobiphenyl/kg body weight in corn oil on days 6, 8, 10, 12, 14 and 16 of pregnancy; 14 control rats were given corn oil (84). The pups were obtained by caesarian on day 19 of pregnancy. No terata were observed and no difference between control, and experimental rats was noted in any of the other parameters studied. Also, five control and six PBB-treated animals were treated with colchicine and bone marrow was studied cytogenetically. Animals treated with PBB and colchicine had higher metaphase and mitotic indices than nontreated animals. Chromosome aberrations were not noted (84).

Toxicity of Chlorinated Naphthalenes

The information on chlorinated naphthalenes is derived from a review published by the EPA (85). For literature references please refer to this report.

Animal Studies

Tables 5–7 summarize the available studies in laboratory animals. Three routes of exposure are reported.

Toxicity in Birds. Penta/hexachloronaphthalenes were studied in the diet of turkey poults. With 5 ppm fed for 40 days a 23% reduction in weight gain with 6.5% mortality was observed; with 100 ppm all poults died within 33 days. Histopathologic examination showed enlarged and darkened livers. The LC_{50} was 20 ppm with an average decrease in weight of 51% (86).

Penta/hexachloronaphthalene in the diet of chickens caused no effects at dietary levels of 4 and 20 ppm (35 days); 100 ppm prevented egg production (35 days), and 2550 ppm caused 100% mortality (14 days). Effects reported included enlarged fibrous livers, lack of feather pigmentation, and pericardial and peritoneal edema. A fourfold increase in vitamin A in the diet decreased toxic effects (87).

Toxicity in Domestic Animals. Ingestion is the most important route for exposure of domestic animals. Highly chlorinated naphthalene poisoning in cattle is known as bovine hyperkeratosis or X-

Table 5. Dietary exposure.

Chlorinated naphthalene	Species	Dose level	Exposure time, days	Effect
Di-	Rat	5 g/kg feed	15	Increase in liver weight, growth impaired, rough coat
Tri-	Mouse	2.5 mg/day	20	None
Tri-	Rat	300 mg/day	9–136	Fatty infiltration of liver
Tri/tetra	Rabbit	15 mg/kg body weight	60	None
Penta-	Rat	50 mg/day	63	Jaundice/fatty degeneration of liver
Penta/hexa-	Rat	300 mg/day	33	Death with yellow livers and extreme fatty degeneration
Penta/hexa-	Rat	100 mg/day	55	Death with yellow livers and extreme fatty degeneration
Penta-	Guinea pig	2.5 mg/kg in oil	48	Death; severe weight loss fatty degeneration of liver
Hexa-	Rat	20 mg/kg	84	Weight loss
Hexa-	Rat	63 mg/kg	84	Weight loss
Hexa-	Rat	200 mg/kg	84	Death
Octa-	Rabbit	1 g	Single dose	Death in 7 days
Octa-	Rat	0.5, 2 or 5 g/kg	22	Decrease in liver but not plasma vitamin A

Table 6. Inhalation studies.

Chlorinated naphthalene	Species	Dose, mg/l.	Exposure	Effect
Tri-	Rat	0.05–0.2	2 hr/day for 20 days	No toxic effect
Tri-	Rat	1.31	16 hr/day for 134 days	No toxic effect
Tri-	Rat	10.98	16 hr/day for 102 days	Slight liver discoloration, 5% rats showed increased fatty degeneration
Penta-	Rat	1.16	16 hr/day for 52 days	Jaundice, enlarged liver and 69% mortality

Table 7. Dermal studies.

Chlorinated naphthalene	Species	Dose	Exposure	Effect
Mono-	Rabbit (ear)	90 mg/g acetone	5–7 days	Mild reddening
Mono-	Rabbit (ear)	500 mg/g acetone	5–7 days	Severe reddening, no decrease of sebaceous glands
Tri-	Rat (skin)	Not given	2 hr/day/40–60 days	None reported
Tri-	Mice (skin)	Not given	2 hr/day/40–60 days	None reported
Penta-	Swine (skin)	60 mg/l.	6 days/week–4 weeks	Slight hyperkeratosis
Penta-	Rabbit (ear)	30 mg/g acetone/day	5 days	Mild dermatitis, hair follicular attenuation
Hexa-	Rabbit (ear)	30 mg/g acetone	5 days	Decrease in sebaceous glands

disease. Detailed information on toxic doses is lacking. The penta/hexa mixture at a dose level of 5.5 mg/kg body weight (orally) for 5 days caused a sharp drop in plasma vitamin A by the end of the third day and depressed plasma vitamin A for over 30 days. A single oral dose of 1 mg/kg body weight of hexachloronaphthalene caused mortality within two weeks. The first effect of chloronaphthalene poisoning is an interference with the biotransformation of carotene to vitamin A. The vitamin A deficiency is quickly followed by inflammation of the oral mucosa, lacrimation, excessive salivation, and irregular food consumption. Gross physical effects that develop during the course of disease include a general thickening of the skin caused by over development of the skin's hairy layer with loss of hair (hyperkeratosis). There may be degeneration or irregular growth of the horns. Continued exposure results in anemia, dehydration, loss of weight, fever and death. There may be severe liver damage (88).

No toxic effects were observed in swine at levels of 100 mg/kg body weight of the chlorinated naphthalenes (degree of chlorination was not marked depression of plasma vitamin A, and death occurred at a dose level of 198 mg/kg body weight (89).

Toxic effects in sheep are observed at doses of chlorinated naphthalene 10 times those required to produce toxicity in cattle. Sheep do not show cutaneous hyperkeratosis or an excessive drop in plasma vitamin A as observed in cattle (90).

Effects reported include nasal discharge, weakness, loss of weight, loss of appetite, ascites, necrosis, and cirrhosis of the liver and cardiovascular effects.

Toxicity in Man

The most important route for man's occupational exposure to chlorinated naphthalene is inhalation. Dermal absorption may also occur.

Penta/hexachloronaphthalene at a concentration of 30 mg/g acetone, applied to the backs of human volunteers for a period of six weeks caused typical chloracne (91).

Daily topical application of mono or dichlorinated naphthalene to the human ear in mineral oil suspension for 30 days caused no observable effect. Two clinically distinct but often concurrent syndromes have been described, namely, liver necrosis, and chloracne with itching (92, 93). A number of deaths involving acute atrophy of the liver have been reported; with the course of the disease similar to other forms of severe liver damage (94, 95). Autopsies of fatally exposed workers show severe yellow

atrophy of the liver. Definite data relating to dose response effects are in general lacking. However, two dermal studies with man have been reported (91, 96).

Summary

The degree of toxicity of chlorinated naphthalenes increases with the degree of chlorination. The higher chlorinated naphthalenes (especially hexa and penta) have been associated with chloracne and liver damage in man. Dose response effects have not been established.

Cattle are more susceptible to the effects of chlorinated naphthalene than other domestic animals. The effects observed include development of vitamin A deficiency, hyperkeratosis, and liver damage.

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